

WHAT IS CLAIMED IS:

1. A method of *ex-vivo* expanding stem and/or progenitor cells, while at the same time, substantially inhibiting differentiation of the stem and/or progenitor cells, the method comprising:

- (a) obtaining a population of cells comprising stem and/or progenitor cells;
- (b) seeding said stem and/or progenitor cells into a bioreactor, and
- (c) culturing said stem and/or progenitor cells *ex-vivo* in said bioreactor under conditions allowing for cell proliferation and, at the same time, culturing said cells under conditions selected from the group consisting of:
 - (i) conditions reducing expression and/or activity of CD38 in said cells;
 - (ii) conditions reducing capacity of said cells in responding to signaling pathways involving CD38 in said cells;
 - (iii) conditions reducing capacity of said cells in responding to retinoic acid, retinoids and/or Vitamin D in said cells;
 - (iv) conditions reducing capacity of said cells in responding to signaling pathways involving the retinoic acid receptor, the retinoid X receptor and/or the Vitamin D receptor in said cells;
 - (v) conditions reducing capacity of said cells in responding to signaling pathways involving PI 3-kinase;
 - (vi) conditions wherein said cells are cultured in the presence of nicotinamide, a nicotinamide analog, a nicotinamide or a nicotinamide analog derivative or a nicotinamide or a nicotinamide analog metabolite;
 - (vii) conditions wherein said cells are cultured in the presence of a copper chelator;
 - (viii) conditions wherein said cells are cultured in the presence of a copper chelate;
 - (ix) conditions wherein said cells are cultured in the presence of a PI 3-kinase inhibitor;

thereby expanding the stem and/or progenitor cells while at the same time, substantially inhibiting differentiation of the stem and/or progenitor cells *ex-vivo*.

107

2. The method of claim 1, wherein said stem and/or progenitor cells are derived from a source selected from the group consisting of hematopoietic cells, umbilical cord blood cells, G-CSF mobilized peripheral blood cells, bone marrow cells, hepatic cells, pancreatic cells, intestinal cells, neural cells, oligodendrocyte cells, keratinocytes, skin cells, muscle cells, bone cells, chondrocytes and stroma cells.

3. The method of claim 1, further comprising the step of selecting a population of stem cells enriched for hematopoietic stem cells.

4. The method of claim 3, wherein said selection is affected via CD34.

5. The method of claim 1, further comprising the step of selecting a population of stem cells enriched for early hematopoietic stem/progenitor cells.

6. The method of claim 5, wherein said selection is affected via CD133.

7. The method of claim 1, wherein step (b) is followed by a step comprising selection of stem and/or progenitor cells.

8. The method of claim 7, wherein said selection is affected via CD 133 or CD 34.

9. The method of claim 1, wherein said providing said conditions for cell proliferation is effected by providing the cells with nutrients and cytokines.

10. The method of claim 9, wherein said cytokines are selected from the group consisting of early acting cytokines and late acting cytokines.

11. The method of claim 10, wherein said early acting cytokines are selected from the group consisting of stem cell factor, FLT3 ligand, interleukin-6, thrombopoietin and interleukin-3.

108

12. The method of claim 10, wherein said late acting cytokines are selected from the group consisting of granulocyte colony stimulating factor, granulocyte/macrophage colony stimulating factor and erythropoietin.

13. The method of claim 10, wherein said late acting cytokine is granulocyte colony stimulating factor.

14. The method of claim 1, wherein said stem and/or progenitor cells are genetically modified cells.

15. The method of claim 1, wherein said inhibitors of PI 3-kinase are wortmannin and/or LY294002.

16. The method of claim 1, wherein said bioreactor is selected from the group consisting of a static bioreactor, a stirred flask bioreactor, a rotating wall vessel bioreactor, a hollow fiber bioreactor and a direct perfusion bioreactor.

17. The method of claim 16, wherein said static bioreactor is selected from the group consisting of well plates, tissue-culture flasks and gas-permeable culture bags.

18. The method of claim 1, wherein said culturing said cells of step (c) is effected in suspension culture.

19. The method of claim 1, wherein said culturing said cells of step (c) is effected on a porous scaffold.

20. The method of claim 19, wherein said porous scaffold is selected from the group consisting of poly (glycolic acid), poly (DL-lactic-co-glycolic acid), alginate, fibronectin, laminin, collagen, hyaluronic acid, Polyhydroxyalkanoate, poly 4 hydroxybuturate (P4HB) and polygluconic acid (PGA).

109

21. The method of claim 19, wherein said porous scaffold comprises a hydrogel.

22. The method of claim 1, wherein said seeding is static seeding or perfusion seeding.

23. The method of claim 1, wherein said culturing of said cells of steps (b) and (c) is effected without stromal cells or a feeder layer.

24. A conditioned medium isolated from the expanded stem and/or progenitor cell culture of claims 1-23.

25. A method of preparing a stem and/or progenitor cell conditioned medium, the method comprising:

(a) establishing a stem and/or progenitor cells culture in a bioreactor according to any of claims 1-23, thereby expanding the stem and/or progenitor cells while at the same time, substantially inhibiting differentiation of the stem and/or progenitor cells *ex-vivo*; and

(b) when a desired stem and/or progenitor cell density has been achieved, collecting medium from said bioreactor, thereby obtaining the stem and/or progenitor cell conditioned medium.

26. The stem and/or progenitor cell conditioned medium of claim 25.

27. A method of transplanting *ex-vivo* expanded stem and/or progenitor cells into a recipient, the method comprising:

(a) obtaining a population of cells comprising stem and/or progenitor cells;
(b) seeding said stem and/or progenitor cells into a bioreactor, and
(c) culturing said stem and/or progenitor cells *ex-vivo* in said bioreactor under conditions allowing for cell proliferation and, at the same time, culturing said cells under conditions selected from the group consisting of:

(i) conditions reducing expression and/or activity of CD38 in said cells;

110

- (ii) conditions reducing capacity of said cells in responding to signaling pathways involving CD38 in said cells;
- (iii) conditions reducing capacity of said cells in responding to retinoic acid, retinoids and/or Vitamin D in said cells;
- (iv) conditions reducing capacity of said cells in responding to signaling pathways involving the retinoic acid receptor, the retinoid X receptor and/or the Vitamin D receptor in said cells;
- (v) conditions reducing capacity of said cells in responding to signaling pathways involving PI 3-kinase;
- (vi) conditions wherein said cells are cultured in the presence of nicotinamide, a nicotinamide analog, a nicotinamide or a nicotinamide analog derivative or a nicotinamide or a nicotinamide analog metabolite;
- (vii) conditions wherein said cells are cultured in the presence of a copper chelator;
- (viii) conditions wherein said cells are cultured in the presence of a copper chelate;
- (ix) conditions wherein said cells are cultured in the presence of a PI 3-kinase inhibitor; and

- (d) recovering said expanded stem and/or progenitor cells from said bioreactor, and
- (e) transplanting into said recipient said *ex-vivo* expanded stem and/or progenitor cells produced in steps (b)- (d).

28. The method of claim 27, wherein said stem and/or progenitor cells are derived from a source selected from the group consisting of hematopoietic cells, umbilical cord blood cells, G-CSF mobilized peripheral blood cells, bone marrow cells, hepatic cells, pancreatic cells, intestinal cells, neural cells, oligodendrocyte cells, skin cells, keratinocytes, muscle cells, bone cells, chondrocytes and stroma cells.

29. The method of claim 27, further comprising the step of selecting a population of stem cells enriched for hematopoietic stem cells.

30. The method of claim 29, wherein said selection is affected via CD34.

111

31. The method of claim 27, further comprising the step of selecting a population of stem cells enriched for early hematopoietic stem/progenitor cells.

32. The method of claim 31, wherein said selection is affected via CD133.

33. The method of claim 27, wherein step (c) is followed by a step comprising selection of stem and/or progenitor cells.

34. The method of claim 33, wherein said selection is affected via CD 133 or CD 34.

35. The method of claim 27, wherein said stem and/or progenitor cells of step (b) are obtained from said recipient.

36. The method of claim 27, wherein said providing said conditions for cell proliferation is effected by providing the cells with nutrients and cytokines.

37. The method of claim 36, wherein said cytokines are selected from the group consisting of early acting cytokines and late acting cytokines.

38. The method of claim 37, wherein said early acting cytokines are selected from the group consisting of stem cell factor, FLT3 ligand, interleukin-6, thrombopoietin and interleukin-3.

39. The method of claim 37, wherein said late acting cytokines are selected from the group consisting of granulocyte colony stimulating factor, granulocyte/macrophage colony stimulating factor and erythropoietin.

40. The method of claim 39, wherein said late acting cytokine is granulocyte colony stimulating factor.

41. The method of claim 27, wherein said stem and/or progenitor cells are genetically modified cells.

42. The method of claim 27, wherein said inhibitors of PI 3-kinase are wortmannin and/or LY294002.

43. The method of claim 27, wherein said bioreactor is selected from the group consisting of a static bioreactor, a stirred flask bioreactor, a rotating wall vessel bioreactor, a hollow fiber bioreactor and a direct perfusion bioreactor.

44. The method of claim 43, wherein said static bioreactor is selected from the group consisting of well plates, tissue-culture flasks and gas-permeable culture bags.

45. The method of claim 27, wherein said culturing said cells of step (c) is effected in suspension culture.

46. The method of claim 27, wherein said culturing said cells of step (c) is effected on a porous scaffold.

47. The method of claim 46, wherein said porous scaffold is selected from the group consisting of poly (glycolic acid), poly (DL-lactic-*co*-glycolic acid), alginate, fibronectin, laminin, collagen, hyaluronic acid, Polyhydroxyalkanoate, poly 4 hydroxybutyrate (P4HB) and polygluconic acid (PGA).

48. The method of claim 41, wherein said porous scaffold comprises a hydrogel.

49. The method of claim 27, wherein said seeding is static seeding or perfusion seeding.

50. The method of claim 27, wherein said culturing of said cells of steps (b) and (c) is effected without stromal cells or a feeder layer.